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Growth and metabolic characteristics of fastidious meat-derived *Lactobacillus algidus* strains

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Abstract

Lactobacillus algidus is a meat spoilage bacterium often dominating the bacterial communities on chilled, packaged meat. Yet, *L. algidus* strains are rarely recovered from meat, and only few studies have focused on this species. The main reason limiting detailed studies on *L. algidus* is related to its poor growth on the media routinely used for culturing food spoilage bacteria. Thus, our study sought to develop reliable culture media for *L. algidus* to enable its recovery from meat, and to allow subculturing and phenotypic analyses of the strains. We assessed the growth of meat-derived *L. algidus* strains on common culture media and their modifications, and explored the suitability of potential media for the recovery of *L. algidus* from meat. Moreover, we determined whether 12 meat-derived *L. algidus* strains selected from our culture collection produce biogenic amines that may compromise safety or quality of meat, and finally, sequenced *de novo* and annotated the genomes of two meat-derived *L. algidus* strains to uncover genes and metabolic pathways relevant for phenotypic traits observed. MRS agar supplemented with complex substances (peptone, meat and yeast extract, liver digest) supported the growth of *L. algidus*, and allowed the recovery of new *L. algidus* isolates from meat. However, most strains grew poorly on standard MRS agar and on general-purpose media. In MRS broth, most strains grew well but a subset of strains required supplementation of MRS broth with additional cysteine. Supplementation of MRS broth with catalase allowed growth in aerated cultures suggesting that the strains produced hydrogen peroxide when grown aerobically. The strains tested (n=12) produced ornithine from arginine and putrescine from agmatine, and two strains produced tyramine from tyrosine. Our findings reveal that *L. algidus* populations are underestimated if routine culture protocols are applied, and prompt concerns that *L. algidus* may generate tyramine or putrescine in meat or fermented meat products.

Keywords *Lactobacillus algidus*, meat spoilage, biogenic amines, tyramine, putrescine, MRS medium

1. Introduction

Lactobacillus algidus is a cold-adapted lactic acid bacterium originally isolated and described from chilled, vacuum-packaged (VP) beef in Japan (Kato et al., 2000). Following the first reports (Kato et al., 2000; Sakala et al., 2002), *L. algidus* has been recovered from meat in other countries including Belgium (Pothakos et al., 2014), Finland (Vihavainen and Björkroth, 2007) and Norway (Schirmer et al., 2009). More recently, studies using next-generation amplicon sequencing to assess bacterial communities in foods have revealed *L. algidus* to be abundant in fresh meats (Delhalle et al., 2016; Jääskeläinen et al., 2016; Mansur et al., 2019; Stoops et al., 2015) but also in processed meat products, such as fermented meat (Janssens et al., 2012) and raw pork sausages (Fougy et al., 2016). Yet, *L. algidus* has remained a relatively poorly characterized species among the meat-associated lactic acid bacteria (LAB).

L. algidus is considered a potential spoilage bacterium in chilled, packaged meats, and it has been associated with the development of “sour and intense” (Schirmer et al. 2009) or “butyric” (Mansur et al., 2019) off-odors in meats. Apart from these observations, only few investigations have focused on *L. algidus*, and thus, its characteristics and role in the spoilage process are poorly understood. For instance, we lack knowledge whether *L. algidus* produces biogenic amines that could compromise the sensory quality or safety of meat. This concern is supported by several studies proving that certain lactobacilli accumulate undesirable biogenic amines, namely cadaverine, histamine, putrescine and tyramine in foods (Bover-Cid et al., 2008; Masson et al., 1996; Pircher et al., 2007). On the other hand, Mansur et al. (2019) proposed that specific *L. algidus* populations might be beneficial for the quality of meat due to inhibition of other bacteria that would otherwise grow to produce offensive spoilage-related metabolites. However, little is known about how *L. algidus* affects the meat microbiome, and subsequently, for the safety or sensory quality of meat.

Culture-independent surveys have proven that *L. algidus* is often abundant in chilled, packaged fresh meat and meat products. Yet, *L. algidus* strains have rarely been cultured from meat relative to other meat-associated LAB (Jääskeläinen et al., 2016; Nieminen et al., 2011). This is partly due to the inability of

L. algidus to produce colonies at 30 °C (Pothakos et al., 2014; Schirmer et al., 2009), a traditional incubation temperature used for culturing meat-borne LAB. However, the main reason why *L. algidus* strains have remained underestimated in culture-based surveys is likely the fastidious nature of the strains complicating their culture in the laboratory (Boguta et al., 2014; Schirmer et al., 2009; Vihavainen and Björkroth, 2007). *L. algidus* strains have often been cultured using a protocol for psychrotrophic LAB that includes a nutrient-rich growth medium, namely de Man Rogosa Sharpe (MRS; De Man et al., 1960), and an anaerobic incubation condition with temperature of 25 °C or below (Nieminen et al., 2011; Pothakos et al., 2014; Sakala et al., 2002; Schirmer et al., 2009; Vihavainen and Björkroth, 2007). However, researchers have raised concerns that this protocol underestimates or even fails to detect *L. algidus* in meat (Jääskeläinen et al., 2016; Nieminen et al., 2011). Indeed, published (Boguta et al., 2014; Vihavainen and Björkroth, 2007) and our unpublished observations have shown that *L. algidus* strains often loose their viability after one or two subcultures in MRS media. Whilst there is little data on the growth requirements of *L. algidus* strains, it is unclear whether their poor growth is due to the strains' requirements for growth factors that are insufficient in the MRS medium. On the other hand, others (Jääskeläinen et al., 2016; Nieminen et al., 2011) have suspected that the low recovery of *L. algidus* strains in culture-based analyses was due to the oxidative stress of cells.

The present study aimed to evaluate common culture media and their modifications for culturing meat-derived *L. algidus* strains to find media that allow recovery of new *L. algidus* isolates from meat, as well as further phenotypic characterization of the isolates. We also assessed the capability of *L. algidus* strains to produce biogenic amines, and finally, determined the genome sequences of two meat-derived strains to explore the genetic basis of the observed metabolic and physiological traits of *L. algidus* strains.

2. Materials and methods

2.1 Strains, culture conditions and media

In this study, we used the type strain *L. algidus* DSM 15638^T from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Culture (the DSMZ), and 11 *L. algidus* strains recovered from meat in our previous studies (Table 1). Strains were maintained at – 72 °C in MRS broth (Oxoid, Basingstoke, United Kingdom) with 25 % glycerol as a cryoprotectant. Cultures were grown in MRS broth (pH 6.2), and on MRS agar (pH 6.2; Oxoid) supplemented with 0.05% (w/v) L-cysteine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) prior to autoclaving. Unless otherwise specified, the broth cultures were incubated statically in ambient air at 20 °C for 48 h, and plate cultures were incubated anaerobically in jars with AnaeroGen sachets (Oxoid,) at 20 °C for 72 h.

2.2 Growth and short-term maintenance of *L. algidus* on solid media

2.2.1 MRS agar modifications

Growth of strains was assessed on three modifications of the standard MRS agar at pH 6.2 (De Man et al., 1960): i) MRS with 0.05% L-cysteine-HCl (MRSc) as a reducing agent to support the growth of oxygen sensitive bacteria; ii) MRSc fortified to contain fourfold amount of bacteriological peptone (40.0 g/L; Oxoid), Lab Lemco meat extract (32.0 g/L; Oxoid) and yeast extract (16.0g/L; Oxoid) (MRSc-F) to provide higher amino acid and vitamin concentrations (Verluyten et al., 2004); and iii) MRSc formulated to include 2.5 g/L of liver digest (MRSc-L) to provide growth factors supporting the growth of anaerobic bacteria (Barrett et al., 2011). The composition of each media and their preparation is described in the supplementary material (Table S1). To assess the growth, the strains were cultured in MRS broth, and streaked onto each media to yield isolated colonies. The plates were incubated anaerobically at 20 °C, and monitored at the day 3 and 5 of incubation for colony appearance and size. If colonies with a diameter of 1.0 mm or above had developed on a plate, a single colony was picked and subcultured in fresh MRSc broth (5 mL) to confirm the viability of the culture and to determine if growth can be initiated from a single colony. In addition, the plates showing colonies were transferred in anaerobic jars for subsequent storage at 5 °C. After 10 days of

storage at 5 °C, the viability of the cultures was assessed by inoculating colonies into fresh MRSc broth and monitoring their growth as visual turbidity of the broth.

2.2.2 MRSc-L with ciprofloxacin and vancomycin (MRSc-L-ab)

To develop a medium for targeted isolation of *L. algidus* from meat, ciprofloxacin and vancomycin, i.e. antibiotics to which many *Lactobacillus* spp. including *L. algidus* are resistant, were added to MRSc-L medium to inhibit the growth of *Lactococcus* spp. and *Leuconostoc* spp. often prevailing in vacuum or modified-atmosphere packaged meat (Jääskeläinen et al., 2016). Ciprofloxacin and vancomycin were diluted into distilled water, sterilized by filtration, and added to cooled MRSc-L medium to a final concentration of 7.5 µg/mL for ciprofloxacin and 50 µg/mL for vancomycin. The growth of *L. algidus* strains on MRSc-L-ab was verified by plating liquid cultures onto MRSc-L-ab.

2.2.3 Growth on general purpose media

Growth of strains (Table 1) was assessed on the following solid media: i) Brain Heart Infusion agar (BHI; Oxoid); ii) Colombia blood agar (Oxoid) with 5% sterile defibrinated bovine blood; iii) Plate Count Agar (PCA; Oxoid); iv) Tryptic soy broth agar generated from Tryptic Soy Broth (Merck) and agar-agar, and containing peptones from casein (17 g/L) and soybean meal (3.0 g/L), and glucose (2.5 g/L); and v) Tryptone Soy Agar (Oxoid) containing casein peptone (15.0 g/L) and soybean peptone (5.0 g/L) but no glucose. Cultures in MRS broth were streaked onto each media to yield individual colonies, and the appearance and size of colonies were observed after 3 and 5 days of anaerobic incubation at 20 °C.

2.3 Targeted isolation of *L. algidus* from meat

Vacuum packaged (VP) beef products (n=2) were purchased from a local retailer, and sampled at the expiration date. Samples (25 g) were homogenized and serially diluted in peptone saline diluent (0.1% peptone, 0.85% NaCl), and bacterial numbers determined by plating 0.1 ml of appropriate dilutions onto MRSc-L-ab plates. After anaerobic incubation at 20 °C for 5 days, the number of LAB per gram of samples was calculated from the colonies observed on the plates containing 25-250 colonies. In total, 144 colonies

were randomly picked for further studies, and subcultured in MRS broth and purified by surface-plating on MRSc-L agar. Then, the isolates were inoculated in MRS broth with glucose replaced by gluconate (MRS-Gln; supplementary Table S1), and those failing to grow in MRS-Gln were considered as presumptive *L. algidus* isolates (Kato et al., 2000). The presumptive *L. algidus* isolates, as well as 10 random isolates growing in MRS-Gln broth, were identified using *Hin*dIII ribotyping with probes targeting to the 16S and 23S rRNA genes as described previously (Vihavainen and Björkroth, 2007; Jääskeläinen et al., 2016). In short, DNA was extracted and fragmented with *Hin*dIII, and the fragments separated using agarose gel electrophoresis, and then, transferred onto a nylon filter. The fragments on the filter were hybridized with probes, and the resulting patterns (ribopatterns) were visualized. Finally, ribopatterns were analyzed using the BioNumerics version 5.10 software (Applied Maths, Sint-Martens-Latem, Belgium): patterns were normalized, and compared to those of the type and reference strains in our in-house database by calculating pattern similarities using the Dice coefficient, and creating a dendrogram by the unweighted-pair group method using average linkages (UPGMA).

2.4 Modified MRS broth for strains growing poorly in MRS broth

During the study, we noticed that a subset of presumptive *L. algidus* isolates recovered from VP beef (see 2.3) grew poorly in standard MRS broth. Thus, several liquid culture media were assessed to find a broth that supported the growth of these isolates. Among those, MRS broth supplemented with fourfold amount of peptone, meat extract and yeast extract (MRS-F broth; see supplementary Table S1) promoted their growth. To assess the factors supporting their growth in MRS-F broth but unavailable in the standard MRS, we tested the growth of a subset of six strains in the following MRS broth modifications: i) MRS broth sterilized by filtration instead of autoclaving to assess growth requirement for heat sensitive substances; ii) MRS broth with 0.2% (v/v) RPMI 1640 Vitamins Solution (Sigma-Aldrich) to assess requirements for B-group vitamins such as nicotinic and pantothenic acids, biotin, and B12; iii) MRS broth with Yeast Nitrogen Base Without Amino Acids (Sigma-Aldrich); and iv) MRS broth with L-cysteine-HCl (0.5 g/L) added after autoclaving.

2.5 Effect of catalase on growth of *L. algidus* in MRS broth

To elucidate whether catalase, a hydrogen peroxide scavenger, could improve the growth of *L. algidus*, the growth of ten *L. algidus* strains with or without catalase (Sigma-Aldrich) was assessed in ambient air or under anaerobic conditions using an automated optical-density monitoring system, Bioscreen C (Growth Curves, Helsinki, Finland). Commercial MRS broth (autoclaved, no cysteine) with or without catalase (1000 U/mL) was loaded (300 µl/well) on a Bioscreen honeycomb plate, and inoculated at 0.1% (v/v) with *L. algidus* strains pre-cultured in MRS broth. The cultures were incubated in the Bioscreen C system at 20 °C, and optical density at 600 nm (OD_{600}) was measured every 0.5 hour for 48 h. Two runs were conducted: one in ambient air and another one in an anaerobic cabinet (MK III; Don Whitley Scientific, Ltd., Shipley) containing 85% N_2, 10% CO_2, and 5% H_2. Both runs were conducted with continuous, medium shaking to allow gas exchange between the broth and the environment. Three biological replicates, each in technical triplicates, were tested for each condition. The data obtained was processed in Microsoft Excel: blank values were subtracted, and values for technical triplicates were averaged. For the growth curves, mean OD_{600} values and standard deviation were calculated for the three biological replicates (each in three replicates). For each strain, significant differences in OD_{600} values obtained in MRS broth and MRS+catalase broth were assed at each time point using a Student's *t* test (two-tailed, assuming unequal variances).

2.6 Conversion of amino acids and biogenic amine production

The ability of strains (listed in Table 1) to produce biogenic amines was assessed in two experiments. Arginine deiminase activity was determined in Niven's et al. (1942) broth with 0.5% of L-arginine monohydrochloride (Sigma-Aldrich) wit initial pH of 6.0. The agmatine deiminase, histidine decarboxylase, lysine decarboxylase, ornithine decarboxylase and tyrosine decarboxylase activities were determined in "decarboxylase" broth (pH 5.3 initially) described by Bover-Cid and Holzapfel (Bover-Cid and Holzapfel, 1999). The strains, pre-cultured in the MRS broth, were inoculated in triplicate at 0.1% (v/v) in 3 mL of decarboxylase broth containing either agmatine, histidine, lysine or ornithine at 1% (w/v) final concertation or L-tyrosine di-sodium salt at 0.5% (w/v) final concertation. All precursors were from Sigma-Aldrich. In

addition, each strain was inoculated in 3 mL of the basal broth devoid of a precursor to be used as control. Cultures were incubated anaerobically at 20 °C, and the color monitored daily for 5 days. The pH of the culture was measured (Inolab 720, WTW, Weilheim, Germany) after 5 days. Test cultures turning to purple and with a pH above 6.0 were considered positive for biogenic amine formation (Bover-Cid and Holzapfel, 1999). Test cultures remaining yellow and with a final pH of 5.0 or below were considered metabolically active but negative for biogenic amine production (Bover-Cid and Holzapfel, 1999).

2.7 Genome sequencing and annotation, and data availability

For the strains LTS37-1 and Nagrm3a-7, DNA was extracted as described previously (Vihavainen and Björkroth, 2007). DNA library for Illumina sequencing was constructed with ~ 300 bp inserts using Nextera XT DNA library Preparation Kit (Illumina, CA, USA). The prepared DNA library was sequenced using Illumina HiSeq 2500 platform for 100 bp paired-end reads. The draft genomes were assembled with Velvet 1.2.08 (Zerbino and Birney, 2008), and annotated using DFAST (Tanizawa et al., 2017). The whole-genome sequence and assembly data were deposited in GenBank under BioProject accession number PRJNA530881 for *Lactobacillus algidus* LTS37-1 and PRJNA531346 for *Lactobacillus algidus* Nagrm3a-7. In addition, the draft genome and assembly data for the type strain *L. algidus* DSM 15638^T under the BioProject number PRJNA224116 were analyzed.

3. Results

3.1 MRS enriched with complex substances allowed colony development of *L. algidus*

Standard MRS agar or MRSc were not suitable for most of the strains (Table 2): 7 of 12 strains assessed, including the type strain *L. algidus* DSM 15638^T, failed to develop separate, good-sized colonies (> 1.0 mm in diameter) on these media. In contrast, all strains grew well on MRS modifications containing increased amounts of nutrient-rich components (Table 2) forming colonies with diameters from 1.2 to 2.0 mm after 3 days of incubation at 20 °C. Regarding viability of the cultures, the colonies picked from MRSc-F and MRSc-L

agar initiated growth when transferred into MRSc and MRS broth. However, based on days to visible turbidity, the growth in MRSc broth was faster than in MRS broth. In MRSc, good growth was visible in 3 days whereas in standard MRS broth without cysteine, good growth was visible only after 4 to 6 days. Regarding short-term maintenance of the strain, the colonies on MRSc-F and MRSc-L agar remained viable for at least for 10 days at 5 °C, and could initiate growth when transferred to MRSc broth.

With regard to the colony formation on general purpose media routinely used in the food microbiology, all 12 *L. algidus* strain failed to produce good-sized colonies on these media (Table 2).

3.2 MRS media modifications allowed recovery of *L. algidus strains from beef*

We tested MRSc-L-ab agar for the enumeration of LAB in VP beef and for targeted isolation of *L. algidus* from meat. In these two samples, we recovered LAB in counts of 5.8×10^6 CFU/g and 6.7×10^6 CFU/g. From the plates of the highest dilutions, we picked 144 colonies (72 isolates for each sample) for further analysis. Of these isolates, 77 (53%) failed to grow in MRS-gcn broth which is a characteristic of *L. algidus* (Kato et al., 2000). Ribotyping of these 77 isolates with *Hin*dIII revealed three distinctive ribopatterns (see Figure S1): the most prevalent one accounted for 58 of 77 isolates, and was identical with the pattern of the type strain *L. algidus* DSM 15638^T (see ribotype "*L. algidus* I" in Figure S1). The second ribotype, accounting for 13 of 77 isolates, was identical with the pattern of the *L. algidus* reference strain Nagrm3a-7. Therefore, we assigned these 72 isolates to *L. algidus*. The remaining six isolates yielded a third ribopattern and were considered as unknown as their pattern did not match with any of those of the type strains in the database (see Ribotype "Unknown" in the Figure S1).

While subculturing the newly isolated *L. algidus* strains, we noticed that 18 (of 72) grew poorly in the standard MRS broth, and thus, required another liquid medium for growth. To obtain liquid cultures, we tested four modifications of MRS broth of which two proved suitable: MRS broth fortified with complex nitrogen sources (MRSc-F broth) and MRS broth supplemented with 0.05% (w/v) L-cysteine added after

autoclaving. However, MRS broth fortified with either vitamin solution or yeast nitrogen base did not support the growth of these strains.

3.3 Catalase improved aerobic and anaerobic growth of *L. algidus*

The strains tested did not grow well in aerated MRS broth (20 °C) without catalase, and we recorded only minor increase in OD_{600}-values during the 48-h incubation period (see Fig. 1 for growth curves of four strains, and supplementary Figure S2 for the other six strains). In contrast, all strains grew well in MRS with catalase (1000 U/mL; Fig. 1 and Fig. S2), and obtained significantly ($p < 0.05$; results not shown) higher OD_{600}-values in MRS with catalase after 4 h of incubation and until the end of the 48-h experiment.

Under anaerobic conditions, all ten strains grew well in MRS broth with and without catalase (see Fig. 2 for growth curves of four strains, and supplementary Figure S2 for other six strains). However, in the presence of catalase (1000 U/mL; Fig. 1 and Fig S2), the lag phase was shorter, and after 7 h of incubation and until the end of the experiment (48 h), the OD_{600} values were significantly ($p < 0.01$; results not shown) higher in cultures with catalase.

3.4 *L. algidus* showed arginine and agmatine deiminase and tyrosine decarboxylase activity

All strains were considered positive for arginine degradation: for all strains the pH of the arginine broth (Niven Jr et al., 1942) was higher at the end of the experiment (mean pH 6.52 ± 0.23 for all) compared to the pH of the control broth devoid of arginine (mean pH 5.88 ± 0.04 for all) and compared to the initial pH of the arginine broth (pH 6.00).

The strains grew also in the Bover-Cid's broth (Bover-Cid and Holzapfel, 1999): the pH decreased in the control broth, and in cultures were histidine, lysine or ornithine was used as a precursor (Fig. 3) suggesting that the strains failed to synthesize histamine from histidine, cadaverin from lysine, or putrescine from ornithine. In contrast, all strains converted agmatine to putrescine, as the pH in agmatine broth increased

(mean pH 7.23 ± 0.16 for all; Fig. 3). In tyrosine broth, the end pH, and thus the end-metabolite produced varied (Fig. 3) among the strains, with only two strains showing tyrosine decarboxylase activity (Fig. 3).

3.5 Genome analysis supported phenotypic observations

All three genomes contained genes that encode enzymes involved in oxygen-dependent metabolism and production of hydrogen peroxide: namely two genes for lactate 2-monooxidase, two for pyruvate oxidase, one alpha-glycerophosphate oxidase (see locus tags in Table S2). In addition, genomes harbored one gene for H_2O-producing NADH oxidase (Table S2). Regarding genes contributing to adaptation to oxidative stress, the genomes harbored thiol peroxidase and glutathione peroxidase (Table S2) but lacked genes for manganese- or heme-dependent catalase, superoxide dismutase or NADH peroxidase. The genomes lacked also key genes related to the respiratory metabolism in LAB, such as cytochrome oxidase operon or ubiquinone/menaquinone biosynthesis methyltransferase.

With regards to amino acid decarboxylases and deiminases, the three genomes harbored genes for a complete agmatine deiminase (AgDI) and arginine deiminase (ADI) pathways (Table S2). In addition, both reference genomes carried the genes for a tyrosine decarboxylase (TDC) route whereas the genome of the type strain lacked this gene cluster (Table S2). We did not identify genes for other amino acid decarboxylases or other pathways implicated in the synthesis of biogenic amines.

4. Discussion

4.1 MRS media modifications supporting growth of *L. algidus*

This study aimed to find a culture medium supporting the growth of *L. algidus* isolates because we suspected that the MRS media commonly used for LAB failed to support the recovery and growth of *L. algidus* strains (Jääskeläinen et al., 2016; Nieminen et al., 2011; Vihavainen and Björkroth, 2007). The results demonstrate that the nutrient concentration of the MRS agar is crucial for the colony formation and size of *L. algidus* (Table 2) with most strains failing to grow on MRS agar unless provided with increased

amount of complex nutrient sources, such as peptone, meat and yeast extracts (MRSc-F agar) or liver digest (MRSc-L).

Notably, in the course of the present work, the type strain *L. algidus* DSM 15638^T failed to form colonies on MRS agar although we followed the culture conditions described in the original species description (Kato et al., 2000) and specified by the culture collection where the type strain was obtained. Consistently, similar difficulties have been reported in previous works where type strain DSM 15638^T failed to grow on MRS media (Boguta et al., 2014; Vihavainen and Björkroth, 2007). Although it is difficult to explain the "unculturability" of the type strain in the present study, we anticipate that it either was due to the stressed DSM 15638^T cells; or, more likely, attributed to the formulation of the MRS agar. The peptone included in the MRS medium is an important source of peptides and amino acids, and the peptone source, or even the batch, may affect bacterial growth (De Man et al., 1960; Gray et al., 2008). Nevertheless, these findings emphasize that the challenges encountered when culturing *L. algidus* strains could be circumvented by adding complex nitrogen sources to MRS agar.

In addition to MRS agar, blood-liver agar (BL agar) is used for culturing fastidious bifidobacteria (Barrett et al., 2011) and also for *L. algidus* (Kato et al., 2000; Sakala et al., 2002). However, dehydrated BL agar, as well as some of its main components, were relatively expensive and not readily available to our laboratory. Thus, we formulated MRSc-L, a medium similar to MRS but supplemented with liver digest (2.5g/L). Liver extract is an important component of BL agar (Barrett et al., 2011), and contains several nurturing components, such as vitamins and reducing agents, enhancing the growth of fastidious anaerobic bacteria. In our study, MRSc-L agar allowed colony development of *L. algidus* strains implying that liver digest or extract, if available, may be used in a culture medium to satisfy the growth requirements of *L. algidus*.

In our study *L. algidus* strains grew poorly on general-purpose media (Table 2) including on Colombia blood agar. Others (Schirmer et al., 2009) have reported that *L. algidus* grows on "blood agar (Oxoid)" implying that the formulation of the blood agar medium, likely the types and amounts of peptones and meat extracts used, affects the growth of *L. algidus.* However, MRS agar is often preferred over blood agar for

culturing LAB, as they store well in pour plates and inhibit the growth of microbes other than LAB decreasing the risk of culture contamination.

4.2 MRS media modifications allowing recovery of *L. algidus* isolates from meat

Our previous works have raised concerns that culture-based assessment of LAB communities using MRS agar underestimates *L. algidus* populations in meat (Jääskeläinen et al., 2016; Nieminen et al., 2011) and that the *L. algidus* strains recovered from meat may only represent a certain subset of *L. algidus* population. In the present study, MRSc-L-ab proved suitable for the recovery of *L. algidus* strains from VP beef but a more comprehensive study is required to assess whether there are subpopulations of *L. algidus* unable to grow on MRSc-L-ab or MRS agar.

Interestingly, 25% (18 of 72) of the *L. algidus* strains recovered from the meat showed fastidious growth requirements. These strains failed to grow in MRS broth (with or without cysteine) and required filter-sterilized MRS broth. While further experiments are necessary to establish which growth factors or nutrients were insufficient in the standard MRS, cysteine (0.05% w/v) added after autoclaving was enough to stimulate the growth of *L. algidus*. Cysteine is often included in the culture media for anaerobic organisms as a reducing agent, and because it improves cells' tolerance to oxidative stress (Wegkamp et al., 2010). Cysteine is essential for many lactobacilli (Saguir and Manca de Nadra, 2007) but the amount required may vary between strains within the same species (Lozo et al., 2008) likely due to differences in function or regulatory mechanisms of amino acid pathways or cysteine uptake (Wegkamp et al., 2010). Although it remains unclear why certain *L. algidus* strains need additional cysteine or fail to grow in standard MRS broth, our observation is of practical importance for future studies aiming to recover and culture LAB isolates from VP meat.

4.3 Catalase benefits *L. algidus* during aerobic culturing

Oxidative stress has been suggested to reduce the recovery potential of *L. algidus* isolates in culture-based studies (Jääskeläinen et al., 2016; Nieminen et al., 2011). In this work, catalase proved necessary for aerobic growth of *L. algidus* in MRS broth (Fig 1 and Fig. S2). This finding suggests that *L. algidus* required catalase to eliminate hydrogen peroxide, a toxic metabolic by-product many lactobacilli accumulate to inhibitory levels in aerated cultures (Martín and Suárez, 2010). Also the analysis of the three *L. algidus* genomes supported this view: we identified genes encoding enzymes that generate hydrogen peroxide and are expressed under aerobic conditions in lactobacilli (De Angelis and Gobbetti, 2004), namely two genes for pyruvate oxidase, two for NADH oxidase and one for alpha-glycerophosphate oxidase. The genomes also encoded genes for hydrogen peroxide scavenging enzymes, such as glutathione peroxidase and thiol peroxidase, but lacked catalases and other enzymes, such as superoxide dismutase or thioredoxin reductase, that are known to scavenge reactive oxygen species in LAB (De Angelis and Gobbetti, 2004). Whilst further studies are required to elucidate the oxidative stress response in *L. algidus,* our results highlight that *L. algidus* cultures are sensitive to oxygen. However, in study settings that require aerated or aerobic culturing, catalase may be added to the growth medium to support the growth of *L. algidus*.

Interestingly, *L. algidus* benefitted from catalase also in anaerobic culture conditions (Fig. 2, Fig S2). However, despite the anaerobic conditions, it is possible, that some oxygen was initially available in the MRS broth leading to hydrogen peroxide accumulation in the beginning of the experiment.

4.4 Deiminase and decarboxylase activity, and genes related to biogenic amine production

In a meat ecosystem, capability to generate biogenic amines and catabolize amino acids is a beneficial adaptation strategy as it provides an alternative way to generate energy and assists in adaptation to low pH (Barbieri et al., 2019). This study demonstrated that *L. algidus* strains produced ornithine from arginine and biogenic amines from agmatine and tyrosine (Table 2). The capability to metabolize tyrosine was, however, strain dependent rather than a general characteristic of *L. algidus* (Table 2). The genome analyses

supported the phenotypic findings, and suggest that *L. algidus* convert agmatine to putrescine via the AgDI pathway, and arginine to ornithine via the ADI pathway. In addition, a tyrosine decarboxylase locus responsible for tyramine formation was predicted in genomes of LTS37-1 and Nagrm3a-7. However, only strain Nagrm3a-7 showed tyrosine decarboxylase activity in Bover-Cid's medium (Table 2) suggesting differences in the gene expression and regulation mechanisms between these two strains. Similarly, (Bargossi et al., 2015) reported that tyraminogenic potential varied widely among *Enterococcus* strains belonging to the same species although all strains possessed a tyrosine decarboxylase gene.

These findings are of significance for food safety as the accumulation of tyramine or putrescine in foods pose a potential health risk, especially for susceptible individuals (Ruiz-Capillas and Jimenez-Colmenero, 2005; ten Brink et al., 1990; Barbieri et al., 2019). In addition, putrescine is a spoilage compound with an offensive, rotten smell, and can be employed as an index of freshness or spoilage in meat (Edwards et al., 1985). In foods, lactobacilli may form tyramine (Eerola et al., 1996) and putrescine from free amino acids or agmatine present the food (Alberto et al., 2007; Ruiz-Capillas and Jiménez-Colmenero, 2004). Additionally, agmatine, a precursor for putrescine, may become available in meat because of activities of *Enterobacteriaceae* able to decarboxylate arginine to agmatine (Alberto et al., 2007; Gänzle, 2015). However, future studies are necessary to determine if *L. algidus* strains form tyramine or putrescin in chilled meat or in fermented meat products, and if the amounts produced are of concern for food safety or quality.

5. Conclusions

This study demonstrated that MRS media used in routine culture protocols fail to support the growth of many *L. algidus* strains whereas these fastidious strains require an enriched modification of MRS medium for growth. The ability of *L. algidus* strains to form putrescine and tyramine warrants further exploration as these activities in meat may compromise the safety or the quality of products. The two novel genome annotations provide a starting point for more detailed studies, such as transcriptomic responses, on

L. algidus, and additionally, for other omics-based studies on meat microbiology. Furthermore, the knowledge gained from this work have critical implications for designing of future studies as robust culture methods are essential for research on this common, yet poorly studied, species.

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FIGURE CAPTIONS

Figure 1. Effect of catalase on aerobic growth of *Lactobacillus algidus.* Bioscreen growth curves of four *L. algidus* strains cultured in MRS broth without (open symbols) and with (solid symbols) catalase (1000 U/mL) aerobically at 20 °C. The values are means with SD of nine replicates. For clarity, only data obtained at 4-h intervals and for 4 strains is shown.

Figure 2. Effect of catalase on anaerobic growth of *Lactobacillus algidus.* Bioscreen growth curves of four *L. algidus* strains cultured in MRS broth without (open symbols) and with (solid symbols) catalase (1000 U/mL) anaerobically at 20 °C. The values are means with SD of nine replicates. For clarity, only data obtained at 4-h intervals and for 4 strains is shown.

Figure 3. Decarboxylase and deiminase activities of *Lactobacillus algidus* strains. The values are end pH obtained for cultures in decarboxylase broth without a precursor amino acid (control) or with agmatine, histidine, lysine, ornithine or tyrosine as a precursor. The initial pH was 5.3; green color indicates decarboxylase/deiminase activity and increased pH, and yellow indicates growth without amine production and decreased pH. Values are mean pH values obtained for 3 replicate cultures measured after 5 days incubation at 20 °C

Table 1. Source information for *Lactobacillus algidus* strains

Strain	Source (country, year)	Reference
2NPPVb12-6	VP[a] beef (Finland, 2015)	(Jääskeläinen et al., 2016)
DSM 15638[T]	VP beef (Japan, not reported)	(Kato et al., 2000; Sakala et al., 2002)
JL6-16	MAP[b] pork and beef (Finland, 2008)	(Nieminen et al., 2011)
JLE1-7	MAP pork and beef (Spain, 2009)	This study
Jlg1-3	MAP pork and beef (Austria, 2008)	This study
Linu2-17	VP beef (Finland, 2009)	This study
LTM35-3	MAP pork (Finland, 2007)	(Fredriksson-Ahomaa et al., 2012)
LTS27-8	MAP pork (Finland, 2007)	(Fredriksson-Ahomaa et al., 2012)
LTS37-1	MAP pork (Finland, 2007)	(Fredriksson-Ahomaa et al., 2012)
Nafim5a-7	MAP beef, marinated (Finland, 2006)	This study
Nagrm3a-7	VP beef, marinated (Finland, 2006)	This study
Nasim5a-2	MAP beef, marinated (Finland, 2009)	This study

[a]VP; vacuum packaged

[b]MAP; modified-atmosphere packaged

Table 2. Growth of twelve *Lactobacillus algidus* strains after 5 days of anaerobic incubation at 20 °C on MRS agar modifications and general-purpose media

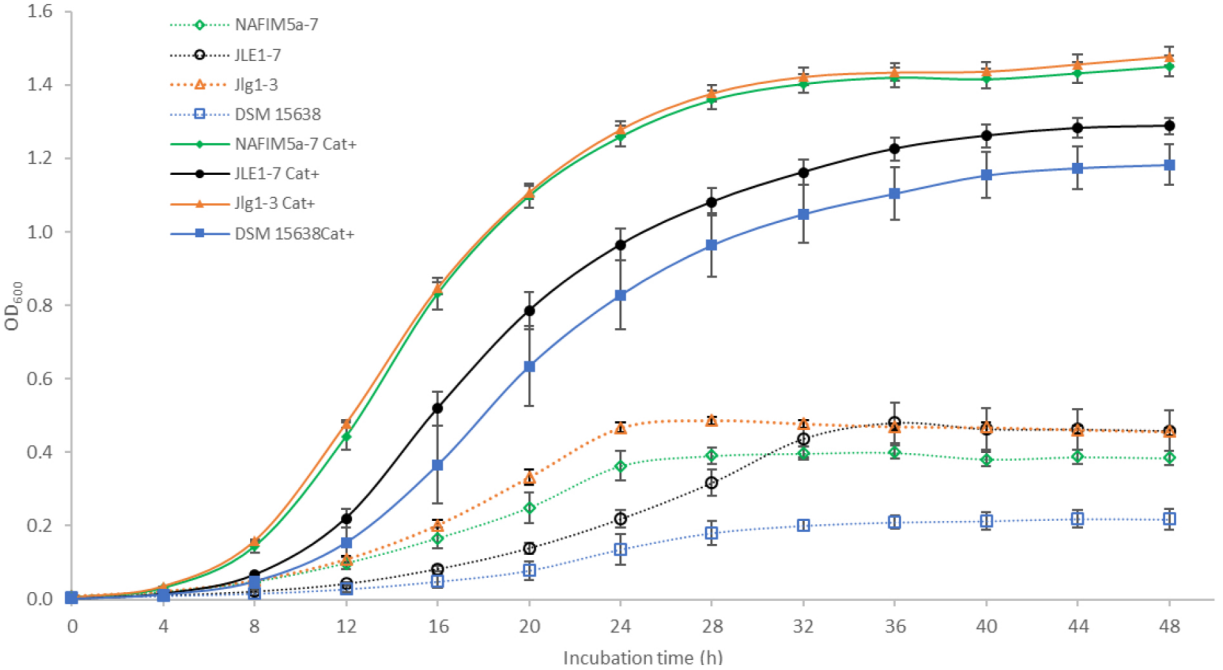
Medium (abbreviation in text)	Motivation	Growth[a]
Standard MRS (MRS)	Routinely used to enumerate and recover lactic acid bacteria (LAB) from meat (De Man et al., 1960)	+/-[b]
MRS with L-cysteine (MRSc)	Routinely used for fastidious anaerobic LAB	+/-[b]
MRSc fortified with complex nitrogen sources (MRSc-F)	To provide increased amount of peptone, meat extract and yeast extract for fastidious LAB (Verluyten et al., 2004)	+
MRSc with liver digest (MRSc-L)	Medium with liver extract has been used to culture *L. algidus* (Kato et al., 2000; Sakala et al., 2002)	+
MRSc-L with ciprofloxacin and vancomycin (MRSc-L-ab)	Increase the probability of isolation of *L. algidus* strains among other LAB in meat	+
Brain heart infusion agar	General purpose medium	(+)
Colombia blood agar	General purpose medium	(+)
Plate count agar	Routinely used for enumeration of bacteria in meat	-
Tryptic soy broth agar	General purpose medium	(+)/-[c]
Tryptone soy agar	General purpose medium	-

[a]Classified based on colony-size: "+" with good-sized (> 1.2 mm) colonies easy to distinct or enumerate; "(+)" with pinpoint colonies difficult to distinct and enumerate; and "-" with no colonies.
[b]Good growth for 5 of 12 strains, weak or no growth for 7 of 12 strains

[C]Weak growth for 5 of 12 strains, no growth for 7 of 12 strains

Highlights

- MRS fortified with complex substances supports the growth of *Lactobacillus algidus*
- Most strains grew poorly on standard MRS and on general-purpose media
- Catalase in MRS broth allowed growth in aerated cultures
- *L. algidus* produced ornithine, putrescine and tyramine *in vitro*
- Genome analysis supported results obtained for biogenic amine production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JLE1-7
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DSM 15638
NAFIM5a-7 Cat+
JLE1-7 Cat+
Jlg1-3 Cat+
DSM 15638Cat+
OD600
Incubation time (h)
0.0
0.2
0.4
0.6
0.8
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48

Figure 1

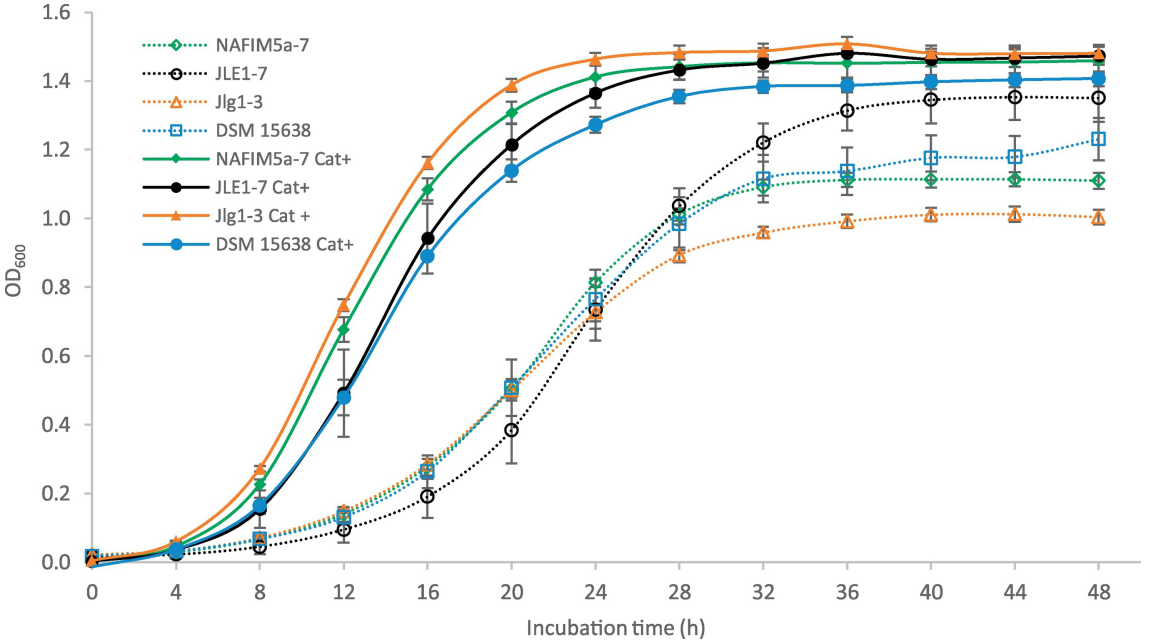
NAFIM5a-7
JLE1-7
Jlg1-3
DSM 15638
NAFIM5a-7 Cat+
JLE1-7 Cat+
Jlg1-3 Cat +
DSM 15638 Cat+
OD600
Incubation time (h)
0.0
0.2
0.4
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Figure 2

Strain	Control	Agmatine	Histidine	Lysine	Ornithine	Tyramine
2NPPVb12-6	4.92	7.17	4.93	4.75	4.74	4.74
DSM 15638^T	4.88	7.48	4.89	4.71	4.70	4.67
JL6-16	4.93	7.22	4.96	4.77	4.77	4.76
JLE1-7	4.94	6.97	4.98	4.79	4.78	4.75
Jlg1-3	4.92	7.37	4.91	4.73	4.74	4.77
Linu2-17	4.93	7.21	4.89	4.76	4.78	4.76
LTM35-3	4.93	7.05	4.96	4.78	4.78	4.76
LTS27-8	4.90	7.45	4.89	4.73	4.74	4.76
LTS37-1	4.88	7.11	4.91	4.75	4.74	4.78
Nafim5a-7	4.91	7.21	4.92	4.73	4.72	4.77
Nagrm3a-7	4.90	7.03	4.92	4.73	4.73	6.16
Nasim5a-2	4.90	7.36	4.92	4.71	4.72	6.16

Figure 3